CLAIMS

1. A process for determining the quantitative and qualitative profile of the repertoire of a given type of an immunoglobulin heavy chain expressed by a lymphocyte B population present in a tissue sample, characterized in that it comprises the following steps:
   (a) obtaining either the cDNA from the mRNA expressed from the tissue sample or the cellular DNA extract of the tissue sample,
   (b) performing the amplification of the cDNA obtained at the step (a) with a set of VH forward primers capable of specifically hybridizing in stringent conditions with the nucleic acids encoding the variable segments (VH) of immunoglobulin heavy chains, said variable segments being distributed among VH subgroups, associated with a CH reverse primer, or a mixture thereof, capable of specifically hybridizing in stringent conditions with the nucleic acid encoding the constant segment (CH) of a given type of an immunoglobulin heavy chain, and
   (c) determining the quantitative and qualitative profile of the repertoire of said type of immunoglobulin heavy chain for each VH subgroup.

2. The process for determining the quantitative and qualitative profile according to claim 1, characterized in that separated amplifications are performed for each of the VH subgroups.

3. The process for determining the quantitative and qualitative profile according to claim 2, characterized in that the separated amplifications are real-time separated amplifications, said real-time amplifications being performed using a CH labeled reverse probe, preferably a CH labeled reverse hydrolysis-probe, capable of specifically hybridizing in stringent conditions with the constant segment of the given type of immunoglobulin heavy chain and capable of

emiting a detectable signal everytime each amplification cycle occurs, and characterized in that the signal obtained for each VH subgroup is measured.

4. The process for determining the quantitative and qualitative profile according to claim 2 or 3, characterized in that the separated amplification products obtained for each of the VH subgroups are further elongated using a CH labeled reverse probe capable of specifically hybridizing in stringent conditions with the constant segment of the given type of immunoglobulin heavy chain and capable of emiting a detectable signal, and characterized in that the elongation products are separated, for each of the VH subgroups, relative to their length, the signal obtained for the separated elongation products is measured, and the quantitative and qualitative profile of the labeling intensity relative to the elongation product length is established, for each of the VH subgroups individually.

5. The process for determining the quantitative and qualitative profile according to anyone of claims 1 to 4, characterized in that the set of VH forward primers comprises at least the 8 following subgroups of VH primers corresponding to the VH subgroups :

- the VH1 primers having the sequences SEQ ID N° 1 to SEQ ID N° 3, and
- the VH2 primer having the sequence SEQ ID N° 4, and
- the VH3a primers having the sequences SEQ ID N° 5 and SEQ ID N° 6, and
- the VH3b primers having the sequences SEQ ID N° 7 to SEQ ID N° 10, and
- the VH4 primers having the sequences SEQ ID N° 11 and SEQ ID N° 12, and
- the VH5 primer having the sequence SEQ ID N° 13, and
- the VH6 primer having the sequence SEQ ID N° 14, and
- the VH7 primer having the sequence SEQ ID N° 15.

6. The process for determining the quantitative and qualitative profile according to claim 5, characterized in that the sequences SEQ ID N° 1 to SEQ ID N° 15

may contain at least one to three point mutations, except for the nucleotides 1 to 6 of their 3' part.

7. The process for determining the quantitative and qualitative profile according to claim 1 to 6, characterized in that the CH reverse primer is selected from the CH reverse primers capable of specifically hybridizing in stringent conditions with the nucleic acids encoding the constant segments (CH) of the IgM heavy chain, the IgE heavy chain, the IgG heavy chain and the IgA heavy chain.

8. The process for determining the quantitative and qualitative profile according to claim 7, characterized in that, when the CH reverse primer is capable of specifically hybridizing in stringent conditions with the nucleic acid encoding the constant segment (CH) of the IgM heavy chain, the CH reverse primer has the sequence SEQ ID N° 26, or the sequence SEQ ID N° 26 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

9. The process for determining the quantitative and qualitative profile according to claim 7, characterized in that, when the CH reverse primer is capable of specifically hybridizing in stringent conditions with the nucleic acid encoding the constant segment (CH) of the IgE heavy chain, the CH reverse primer has the sequence SEQ ID N° 33, or the sequence SEQ ID N° 33 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

10. The process for determining the quantitative and qualitative profile according to claim 7, characterized in that, when the CH reverse primer is capable of specifically hybridizing in stringent conditions with the nucleic acid encoding the constant segment (CH) of the IgE heavy chain, the CH reverse primer has the sequence SEQ ID N° 42, or the sequence SEQ ID N° 42 wherein one to

three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

11. The process for determining the quantitative and qualitative profile according to claim 7, characterized in that, when the given type of immunoglobulin heavy chain is the IgG type, a mixture of two CH reverse primers is associated with the set of VH forward primers,
said two CH reverse primers having the sequences SEQ ID N° 27 and SEQ ID N° 28, or the sequences SEQ ID N° 27 and SEQ ID N° 28 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

12. The process for determining the quantitative and qualitative profile according to claim 7, characterized in that, when the CH reverse primer is capable of specifically hybridizing in stringent conditions with the nucleic acid encoding the constant segment (CH) of the IgG heavy chain, the sequence of the CH reverse primer is selected from the group consisting of SEQ ID N°40 and SEQ ID N°41, or SEQ ID N° 40 and SEQ ID N°41 wherein one to tree point mutations may occur, except for the nucleotides 1 to 6 of their 3' part.

13. The process for determining the quantitative and qualitative profile according to claim 12, wherein the sequence of the CH reverse primer is SEQ ID N°41.

14. The process for determining the quantitative and qualitative profile according to claim 7 or 8, characterized in that, when the given type of immunoglobulin heavy chain is an IgM heavy chain and when the separated amplifications are real-time separated amplifications, the CH labeled hydrolysis-probe has the sequence SEQ ID N° 29, or the sequence SEQ ID N° 29 wherein at least one point mutation may occur.

15. The process for determining the quantitative and qualitative profile according to claim 7, 8 or 14, characterized in that, when the given type of immunoglobulin heavy chain is an IgM heavy chain and when the separated amplification products obtained for each of the VH subgroups are further elongated, the CH labeled reverse probe has the sequence SEQ ID N° 30, or the sequence SEQ ID N° 30 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

16. The process for determining the quantitative and qualitative profile according to claim 7 or 9, characterized in that, when the given type of immunoglobulin heavy chain is an IgE heavy chain, when the CH reverse primer has the sequence SEQ ID N°33, and when the separated amplifications are real-time separated amplifications, the CH labeled hydrolysis-probe has the sequence SEQ ID N° 36, or the sequence SEQ ID N° 36 wherein at least one point mutation may occur.

17. The process for determining the quantitative and qualitative profile according claim 7 or 10, characterized in that, when the given type of immunoglobulin heavy chain is an IgE heavy chain, when the CH reverse primer has the sequence SEQ ID N°42, and when the separated amplifications are real-time separated amplifications, the CH labeled hydrolysis probe has the sequence SEQ ID N°43, or the sequence SEQ ID N°43 wherein at least one point mutation may occur.

18. The process for determining the quantitative and qualitative profile according to claim 7, 9, 10, 16 or 17, characterized in that, when the given type of immunoglobulin heavy chain is an IgE heavy chain and when the separated amplification products obtained for each of the VH subgroups are further elongated, the CH labeled reverse probe has the sequence SEQ ID N° 37, or the sequence SEQ ID N° 37 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

19. The process for determining the quantitative and qualitative profile according to claim 11, 12 or 13, characterized in that, when the given type of immunoglobulin heavy chain is an IgG heavy chain and when the separated amplifications are real-time separated amplifications, the CH labeled hydrolysis-probe has the sequence SEQ ID N° 34, or the sequence SEQ ID N° 34 wherein at least one point mutation may occur.

20. The process for determining the quantitative and qualitative profile according to claim 7, 11, 12, 13 or 19, characterized in that, when the given type of immunoglobulin heavy chain is an IgG heavy chain and when the separated amplification products obtained for each of the VH subgroups are further elongated, the CH labeled reverse probe has the sequence SEQ ID N° 35, or the sequence SEQ ID N° 35 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

21. The process for determining the quantitative and qualitative profile according to claim 7, characterized in that, when the CH reverse primer is capable of specifically hybridizing in stringent conditions with the nucleic acid encoding the constant segment (CH) of the IgA heavy chain, the CH reverse primer has the sequence SEQ ID N° 44, or the sequence SEQ ID N° 44 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

22. The process for determining the quantitative and qualitative profile according to claim 7 or 21, characterized in that, when the given type of immunoglobulin heavy chain is an IgA heavy chain and when the separated amplifications are real-time separated amplifications, the CH labeled hydrolysis-probe has the sequence SEQ ID N° 46, or the sequence SEQ ID N° 46 wherein at least one point mutation may occur.

23. The process for determining the quantitative and qualitative profile according to claim 7, 21 or 22, characterized in that, when the given type of immunoglobulin heavy chain is an IgA heavy chain and when the separated amplification products obtained for each of the VH subgroups are further elongated, the CH labeled reverse probe has the sequence SEQ ID N° 45, or the sequence SEQ ID N° 45 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

24. The process for determining the quantitative and qualitative profile according to anyone of claims 2 to 20, characterized in that further separated amplifications for each of JH subgroups are performed from the separated amplification products obtained for at least one given VH subgroup of the VH subgroups with the CH reverse primer,
said further separated amplifications being performed using a VH internal forward primer corresponding to the given VH subgroup, and associated with a set of JH reverse primers corresponding to the JH subgroups and capable of specifically hybridizing in stringent conditions with the nucleic acids encoding the junction segments of the given type of immunoglobulin heavy chain.

25. The process for determining the quantitative and qualitative profile according to claim 24, characterized in that the further separated amplifications are real-time amplifications performed using a VH labeled forward probe, preferably a VH labeled forward hydrolysis-probe, capable of specifically hybridizing in stringent conditions with the variable segment of the given type of immunoglobulin heavy chain and capable of emiting a detectable signal everytime each amplification cycle occurs, and characterized in that the signal obtained for each JH subgroup is measured.

26. The process for determining the quantitative and qualitative profile according to claim 24 or 25, characterized in that, when the given VH subgroup is the VH5 subgroup, the VH5 internal forward primer has the sequence SEQ ID N°

31, or the sequence SEQ ID N° 31 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

27. The process for determining the quantitative and qualitative profile according to claim 24 or 25, characterized in that, when the given VH subgroup is the VH4 subgroup, the VH4 internal forward primer has the sequence SEQ ID N°47, or the sequence SEQ ID N°47 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

28. The process for determining the quantitative and qualitative profile according to claim 26 or 27, characterized in that the VH labeled forward hydrolysis-probe has the sequence SEQ ID N° 32, or the sequence SEQ ID N° 32 wherein at least one point mutation may occur.

29. The process for determining the quantitative and qualitative profile according to anyone of claims 2 to 20, characterized in that separated elongations are performed for each of the JH subgroups from the separated amplification products obtained for at least one given VH subgroup of the VH subgroups with the CH reverse primer,

said further separated elongations being performed using a set of JH labeled reverse primers corresponding to JH subgroups and capable of specifically hybridizing in stringent conditions with the nucleic acids encoding the junction segments of the given type of immunoglobulin heavy chain, said JH labeled reverse primers being capable of emiting a detectable signal,

and characterized in that the elongation products are separated, for each of the JH subgroups, relative to their length, the signal obtained for the separated elongation products is measured, and the quantitative and qualitative profile of the labeling intensity relative to the elongation product length is established, for each of the JH subgroups for the given VH subgroup.

30. The process for determining the quantitative and qualitative profile according to anyone of claims 24 to 29, characterized in that the set of JH forward primers, optionally labeled, comprises at least the 6 following subgroups of JH primers corresponding to the JH subgroups :
- the JH1 primer having the sequence SEQ ID N° 16, and
- the JH2 primer having the sequence SEQ ID N° 17, and
- the JH3 primer having the sequence SEQ ID N° 18, and
- the JH4 primers having the sequences SEQ ID N° 19 to SEQ ID N° 21, and
- the JH5 primer having the sequence SEQ ID N° 22, and
- the JH6 primers having the sequences SEQ ID N° 23 to SEQ ID N° 25.

31. The process for determining the quantitative and qualitative profile according to claim 30, characterized in that the sequences SEQ ID N° 16 to SEQ ID N° 25 may contain at least one to three point mutations, except for the nucleotides 1 to 6 of their 3' part.

32. A set of VH forward primers capable of specifically hybridizing in stringent conditions with the nucleic acids encoding the variable segments (VH) of immunoglobulin heavy chains, said variable segments being distributed among at least 8 VH subgroups, associated with a CH reverse primer, or a mixture thereof, capable of specifically hybridizing in stringent conditions with the nucleic acid encoding the constant segment (CH) of a given type of an immunoglobulin heavy chain, characterized in that the set of VH forward primers comprises at least the 8 following subgroups of VH primers corresponding to the VH subgroups :
- the VH1 primers having the sequences SEQ ID N° 1 to SEQ ID N° 3, and
- the VH2 primer having the sequence SEQ ID N° 4, and
- the VH3a primers having the sequences SEQ ID N° 5 and SEQ ID N° 6, and
- the VH3b primers having the sequences SEQ ID N° 7 to SEQ ID N° 10, and
- the VH4 primers having the sequences SEQ ID N° 11 and SEQ ID N° 12, and

- the VH5 primer having the sequence SEQ ID N° 13, and
- the VH6 primer having the sequence SEQ ID N° 14, and
- the VH7 primer having the sequence SEQ ID N° 15.

33. The set of VH forward primers according to claim 32, characterized in that the sequences SEQ ID N° 1 to SEQ ID N° 15 may contain at least one to three point mutations, except for the nucleotides 1 to 6 of their 3' part.

34. The set of VH forward primers according to claim 32 or 33, characterized in that the CH reverse primer is selected from the CH reverse primers capable of specifically hybridizing in stringent conditions with the nucleic acids encoding the constant segments (CH) of the IgM heavy chain, the IgE heavy chain, the IgG heavy chain and the IgA heavy chain.

35. The set of VH forward primers according to claim 34, characterized in that, when the CH reverse primer is capable of specifically hybridizing in stringent conditions with the nucleic acid encoding the constant segment (CH) of the IgM heavy chain, the CH reverse primer has the sequence SEQ ID N° 26, or the sequence SEQ ID N° 26 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

36. The set of VH forward primers according to claim 34, characterized in that, when the CH reverse primer is capable of specifically hybridizing in stringent conditions with the nucleic acid encoding the constant segment (CH) of the IgE heavy chain, the CH reverse primer has the sequence SEQ ID N° 33, or the sequence SEQ ID N° 33 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

37. The set of VH forward primers according to claim 34, characterized in that, when the CH reverse primer is capable of specifically hybridizing in stringent conditions with the nucleic acid encoding the constant segment (CH) of the

IgE heavy chain, the CH reverse primer has the sequence SEQ ID N° 42, or the sequence SEQ ID N° 42 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

38. The set of VH forward primers according to claim 34, characterized in that, when the given type of immunoglobulin heavy chain is the IgG type, a mixture of two CH reverse primers is associated with the set of VH forward primers,
said two CH reverse primers having the sequences SEQ ID N° 27 and SEQ ID N° 28, or the sequences SEQ ID N° 27 and SEQ ID N° 28 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.

39. The set of VH forward primers according to claim 34, characterized in that, when the CH reverse primer is capable of specifically hybridizing in stringent conditions with the nucleic acid encoding the constant segment (CH) of the IgG heavy chain, the sequence of the CH reverse primer is selected from the group consisting of SEQ ID N°40 and SEQ ID N°41, or SEQ ID N°40 or SEQ ID N°41 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of their 3' part.

40. The process for determining the quantitative and qualitative profile according to claim 39, wherein the sequence of the CH reverse primer is SEQ ID N°41.

41. A method for the *in vitro* diagnosis of a condition associated with an abnormal expression of the repertoire of a given type of an immunoglobulin heavy chain by a lymphocyte B population in a subject, characterized in that it comprises the following steps:

(1) determining the quantitative and qualitative profile of the given type of immunoglobulin heavy chain from a tissue sample of said subject according to anyone of claims 1 to 31,

(2) comparing the quantitative and qualitative profile obtained at the step (1) with a control quantitative and qualitative profile of said given type of immunoglobulin heavy chain, the demonstration of a significant modification of the profile obtained at the step (1) being significant of such a condition in the subject.

42. The method for the *in vitro* diagnosis according to claim 41, characterized in that the condition is an auto-immune disease, a B cell lymphoma or an immunodepressive disease.

43. The method for the *in vitro* diagnosis according to claim 41, characterized in that the condition results from a bone marrow transplantation, from a vaccinal test or from an allergic reaction.

44. A method for the *in vitro* follow-up of a treatment of a condition associated with an abnormal expression of the repertoire of a given type of an immunoglobulin heavy chain by a lymphocyte B population in a subject, characterized in that it comprises the following steps:

(1) optionally, determining before the treatment the quantitative and qualitative profile of the given type of immunoglobulin heavy chain from a tissue sample of said subject according to anyone of claims 1 to 26,

(2) determining, during the treatment, the quantitative and qualitative profile of the given type of immunoglobulin heavy chain at given times from tissue samples of said subject according to anyone of claims 1 to 26,

(3) comparing the quantitative and qualitative profiles obtained at the step (2) and optionally at the step (1) with each others and optionally with a control quantitative and qualitative profile of the given type of immunoglobulin heavy chain, the demonstration of a significant modification of the profile obtained at the step (1) being significant of such a condition in the subject.

45. The method for the *in vitro* follow-up according to claim 44, characterized in that the condition is an auto-immune disease, a B cell lymphoma or an immunodepressive disease.

46. The method for the *in vitro* follow-up according to claim 44, characterized in that the condition results from a bone marrow transplantation, from a vaccinal test or from an allergic reaction.

47. A kit for determining the quantitative and qualitative profile of the repertoire a given type of an immunoglobulin heavy chain expressed by a lymphocyte B population present in a tissue sample, characterized in that it comprises the set of VH forward primers according to anyone of claims 32 to 38 associated with the CH reverse primer.

48. The kit according to claim 47, characterized in that it further comprises a set of JH reverse primers, optionally labeled, corresponding to the JH subgroups and capable of specifically hybridizing in stringent conditions with the nucleic acids encoding the junction segments of the given type of immunoglobulin heavy chain.

49. The kit according to claim 48, characterized in that the set of JH reverse primers comprises the 6 following subgroups of JH primers corresponding to the JH subgroups :

- the JH1 primer having the sequence SEQ ID N° 16, and
- the JH2 primer having the sequence SEQ ID N° 17, and
- the JH3 primer having the sequence SEQ ID N° 18, and
- the JH4 primers having the sequences SEQ ID N° 19 to SEQ ID N° 21, and
- the JH5 primer having the sequence SEQ ID N° 22, and
- the JH6 primers having the sequences SEQ ID N° 23 to SEQ ID N° 25.

50. The kit according to claim 49, characterized in that the sequences SEQ ID N° 16 to SEQ ID N° 25 may contain at least one to three point mutations, except for the nucleotides 1 to 6 of their 3' part.

51. Use of the kit according to anyone of claims 47 to 50, for the *in vitro* diagnosis of a condition associated with an abnormal expression of the repertoire of a given type of an immunoglobulin heavy chain by a lymphocyte B population in a subject.

52. The use of the kit according to claim 51, characterized in that the condition is an auto-immune disease, a B cell lymphoma or an immunodepressive disease.

53. The use of the kit according to claim 52, characterized in that the condition results from a bone marrow transplantation, from a vaccinal test or from an allergic reaction.

54. The set of VH forward primers according to claim 34, characterized in that, when the CH reverse primer is capable of specifically hybridizing in stringent conditions with the nucleic acid encoding the constant segment (CH) of the IgA heavy chain, the CH reverse primer has the sequence SEQ ID N° 44, or the sequence SEQ ID N° 44 wherein one to three point mutations may occur, except for the nucleotides 1 to 6 of its 3' part.